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Research paper

Association of the Ala16Val MnSOD gene polymorphism with plasma leptin levels and oxidative stress biomarkers in obese patients

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ABSTRACT

Chronic oxidative stress is a major characteristic of obesity. Manganese superoxide dismutase (*MnSOD*) is an antioxidant enzyme known to be present within mitochondria and is considered a main defense against oxidative stress. The aim of this study was to investigate the association between the *MnSOD* gene Ala16Val polymorphism in obesity in terms of body mass index (BMI), lipid parameters, plasma leptin levels, homeostasis model assessment of insulin resistance (HOMA-IR), and oxidative stress biomarkers. The study included 150 obese and 120 non-obese subjects. The *MnSOD* Ala16Val polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Plasma leptin levels, serum lipid, superoxide dismutase (SOD), malondialdehyde (MDA), and anthropometric parameters were measured. No association was found between the *MnSOD* gene Ala16Val polymorphism and BMI in the study or control group. Strikingly, in the study group, obese subjects with the VV genotype had significantly higher plasma leptin levels ($p < 0.001$) than those with the AA and AV genotypes. Serum total cholesterol ($p < 0.01$) and MDA ($p < 0.001$) levels were significantly higher in subjects with the VV genotype for *MnSOD* in the obese and non-obese groups. In the obese group, subjects with the VV genotype had significantly lower SOD ($p < 0.001$) activity than the AA and AV genotypes. Our results suggest that the *MnSOD* gene polymorphism was associated with leptin levels and superoxide dismutase activity in the obese group but had no direct association with obesity. Moreover, the Ala16Val polymorphism has a significant effect on lipid profiles and MDA levels in both obese and non-obese subjects.

1. Introduction

Obesity, a growing health problem worldwide, is well known for its role in the increased incidence of various diseases, including metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and some cancers. Various mechanisms underlying the association of each disease with obesity have been proposed. A common issue in the pathogenesis of many obesity-associated diseases is oxidative stress (Matsuda and Shimomura, 2013). There are several possible contributors to oxidative stress in obesity, including hyperglycemia, elevated tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, chronic inflammation, and hyperleptinemia (Vincent and Taylor, 2006).

Several reports on the role of enzymes related to oxidative metabolism, such as superoxide dismutases (SODs), have been published recently (Karaouzene et al., 2011; Shih et al., 2006). SODs catalyze the dismutation of reactive superoxide radicals to hydrogen peroxide in mammals. Three isoforms of SOD have been identified, including the manganese SOD (MnSOD) in mitochondria (Zelko et al., 2002). MnSOD is encoded by a single gene containing five exons, located at 6q25 (Shimoda-Matsubayashi et al., 1996). Several single nucleotide polymorphisms (SNPs) have been described in the *MnSOD* gene, one of which is Ala16Val. The change of alanine (Ala) to valine (Val) at the 16th amino acid (Ala16Val) of the signal sequence of *MnSOD* (nine amino acids from the first amino acid of the mature protein) has been suggested to change the secondary structure of the protein and therefore the mitochondrial targeting of the enzyme (Rosenblum et al., 1996). The precursor sequence in the MnSOD protein is known as the mitochondrial target sequence (MTS). Sutton et al. (2005) suggested that the Ala-MnSOD precursor generated 30–40% more of the active, matricial, and processed *MnSOD* homotetramer than the Val-MnSOD precursor.

Abbreviations: MnSOD, Manganese superoxide dismutase; BMI, Body mass index; HOMA-IR, Homeostasis model assessment of insulin resistance; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; SOD, superoxide dismutase; MDA, Malondialdehyde; SNPs, Single nucleotide polymorphisms; MTS, Mitochondrial target sequence; Ala, Alanine; Val, Valine; TG, Triglycerides; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; ELISA, Enzyme-linked immunosorbent assay; ANOVA, Analysis of variance; SD, Standard deviation; ROS, Reactive oxygen species.

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Among epidemiological investigations of a potential link between the *MnSOD* Ala16Val genotype and over 30 diseases and disorders, almost half reported some relationship (Crawford et al., 2012). Several investigations have suggested that the Ala allele, thought to alter enzyme transportation into mitochondria, is associated with breast, prostate and colon cancer, hepatocellular carcinoma in hepatitis C virus-infected patients, and malignant pleural mesothelioma (Slanger et al., 2006; Ezzikouri et al., 2008; Kang et al., 2007; Landi et al., 2007). Surprisingly, most studies have shown that the Val allele is positively associated with cardiovascular disease risk and with comorbidities in type 1 diabetes mellitus and type 2 diabetes mellitus patients (Nakanishi et al., 2008; Möllsten et al., 2007; Chen et al., 2012). Although the associations of the *MnSOD* gene Ala16Val polymorphism in cancers and cardiovascular disease have been investigated extensively, studies involving obese subjects are limited. Montano et al. (2009) reported an association between the Ala16Val polymorphism and obesity.

The evidence for an association between obesity and superoxide production involves mainly levels of hormones and molecules related to fat tissue and energy metabolism, such as leptin. Therefore, the association between the Ala16Val polymorphism of the *MnSOD* gene and obesity may involve modulation of these molecules. However, there is no reported study addressing the relationship between the *MnSOD* polymorphism and leptin levels in obesity. Thus, the aim of this study was to investigate the associations between the *MnSOD* gene Ala16Val polymorphism, BMI, lipid parameters, HOMA-IR, lipid peroxidation, antioxidant enzyme activity, and plasma leptin levels in obese subjects versus non-obese subjects.

2. Materials and methods

2.1. Subjects

This prospective study enrolled patients who attended the outpatient clinic of the Endocrinology Department at Famagusta Government Hospital, who were divided into two groups. One group consisted of 150 obese patients having a mean age of 40.06 ± 8.74 years and BMI of 35.05 ± 6.07 kg/m^2. The second group consisted of 120 non-obese subjects. The mean age of these subjects was 38.8 ± 8.21 years and their mean BMI was 22.33 ± 2.08 kg/m^2. None of the participants had hypertension, liver, kidney, thyroid, cardiovascular, or any active inflammatory disease and they were questioned about any medical therapy that might affect lipid and glucose metabolism. The participants neither received any medications nor participated in any dietary or exercise program during the study. All subjects provided written informed consent before enrolment. The Near East University Research Ethics Committee approved the study.

2.2. Anthropometric measurements

All measurements were performed in the morning with the patients in a fasting state and anthropometric measurements, including weight (kg), height (m), hip circumference (cm) and waist circumference (cm) for each subject were measured barefoot and lightly clothed. Hip circumference was measured by placing a tape measure around the patient's hips at the level of the prominences over the greater trochanters of both femurs. Waist circumference was measured midway between the lowest rib (laterally) and the iliocristale landmark with flexible tape. BMI was calculated as body weight (kg) divided by the square of height (m^2) and obesity was defined as a BMI ≥ 30 kg/m^2 (World Health Organization, 1995).

2.3. Biochemical parameters

Blood samples were obtained after an overnight fast. Levels of serum glucose, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using an automated clinical chemistry analyzer (Abbott Architect C8000). Fasting insulin concentrations were measured using an electrochemiluminescence kit (Ref. 12017547; Elecsys Corp., Lenexa, KS). Insulin resistance index values were calculated using the homeostasis model assessment of insulin resistance ('HOMA-IR'), as the product of fasting insulin (μU/mL) and fasting glucose (mmol/L) divided by 22.5 (Matthews et al., 1985).

Serum MDA levels were determined according to the method of Buerge and Aust and are expressed as nmol/mL (Buerge and Aust, 1978). Whole blood superoxide dismutase activity was measured as described by Mc Cord and Fridovich (1969). Plasma leptin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) human leptin kit (EIA-2395; DRG International, Inc., USA). Human leptin kits were used according to the manufacturer's protocol. The results are expressed in ng/mL.

2.4. MnSOD gene Ala16Val polymorphism

Genomic DNA was extracted from whole blood by salting out procedure (Miller et al., 1988). Genotyping of the *MnSOD* gene Ala16Val polymorphism (rs4880) was carried out using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay with previously described primer pairs (Gottlieb et al., 2005). PCR reactions were performed on a total volume of 50 μl using 1 μg of genomic DNA, 0.4 μM of each primer, 23.5 μl nuclease-free water (Fermentas International Inc, Canada) and 25 μl DreamTaq PCR Master Mix (Fermentas International Inc, Canada). PCR consisted of one cycle of initial denaturation for 2 min at 95 °C, followed by 32 cycles denaturation for 30 s at 95 °C, annealing for 30 s at 54 °C and extension for 30 s at 72 °C and a final extension at 72 °C for 10 min. PCR products were digested for 2 h at 37 °C with 5 U of *HhaeIII* restriction enzyme (15U; at 37 °C, for 6 h, Gibco. Inc, Co). Digest products (23 and 85 bp) were visualized on a 6% agarose gel stained with ethidium bromide. A mutation was introduced by a primer mismatch to a create a restriction cut site for *HhaeIII* in the −9 codon so that the following genotypes were observed: −9 Ala/Ala (23 and 85 bp), −9 Ala/Val (23, 85 and 110 bp), and −9 Val/Val (110 bp).

2.5. Statistical analysis

The distributions of continuous variables in groups were expressed as means ± standard deviation (SD). Differences in baseline characteristics between groups were analyzed by Student's *t*-test and the χ^2 test for continuous variables and categorical variables, respectively. Analysis of variance (ANOVA) was used to compare means of continuous variables in the three genotype subgroups. The differences in the mean values of continuous variables in the three genotype subgroups were confirmed by a post hoc Tukey test. A P value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed using the SPSS software (ver. 15.0; SPSS Inc., Chicago, IL).

3. Results

Descriptive statistics of anthropometric and metabolic characteristics of the study population are presented in Table 1. Obese and non-obese subjects did not differ in age, while plasma glucose, total cholesterol, triglycerides, LDL-cholesterol, leptin, and MDA levels ($p < 0.001$) were significantly higher and mean HDL-cholesterol ($p < 0.001$) levels were significantly lower in obese than non-obese subjects. Non-obese subjects had significantly higher SOD levels and lower HOMA-IR than obese subjects ($p < 0.001$).

Analysis of the *MnSOD* gene yielded three variants of the genotype: AA (wild-type), AV (heterozygous), and VV (homozygous). The Mn-SOD genotype frequencies were calculated and are presented in Table 2 and Fig. 1. In obese subjects, the genotype frequencies were 28.66% for AA, 31.33% for AV, and 40% for VV. The frequencies of AA,